Last updated: 29-Dec-11

# Sample Requirements for "*Run*"

## 1. Sequencing Reaction

Our system is set up to cope with sequencing reactions of varying quality and intensity. However, the best cost/quality ratio can be obtained by setting up reactions in a total volume of 10 µl with 0.5 µl of BigDye Terminator Sequencing Mix (either v1.1 or v3.1). The "*Run*" service is available only for full 96-well plates.

## 2. Reaction purification

For best results and to prolong capillary lifetime small molecules (especially salts) and unincorporated dye terminators have to be removed completely prior to electrophoresis. We recommend an SDS/heat treatment to minimize dye blobs followed by purification via Sephadex columns.

## 3. Job submission

Sign up for your job using the "*Run*" service with either BigDye v1.1 or v3.1 on the sequencing homepage (http://www.genetik.biologie.uni-muenchen.de/sequencing). The 96 samples can either be named individually or sample names can be uploaded as a tab-delimited text file for the whole plate at once. For this, arrange sample names in Excel in a 12x8 grid and save this grid accordingly.

## 4. Sample delivery

Deposit your **clearly labeled** samples in the fridge in room G03.031 (Sequencing Service im LMU Biozentrum, Großhaderner Str. 2-4, 82152 Martinsried). If deposited by 10:00 AM they will be processed on the same day. The samples have to be in a half-skirted 96-well plate (can be obtained at the Genomics Service Unit) in a total volume of at least 20 µl of which at least 10 µl should be formamide to stabilize the reaction products. Usually, sequence data can be downloaded from the sequencing homepage on the same day.

## 5. Troubleshooting

The majority of sequencing problems are due to either incorrect template or primer concentration or contaminants in the template. Contaminants known to interfere with the sequencing reaction are: salts (NaCl, NaAc, KAc, KCl), chelators (EDTA, EGTA), proteins, detergents (SDS, Triton X-100), RNA, chromosomal DNA, organic chemicals (ethanol, chloroform, phenol), divalent cations ($Mg^{2+}$, $Ca^{2+}$, $Mn^{2+}$), and excess PCR primers, dNTPs, enzyme, and buffer components from PCR. Be sure to clean your DNA template and check the quantity and quality before sequencing.

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